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Differences between CEM and Human Peripheral Blood T Lymphocytes in cAMP-Dependent HIV Viral Fusion and CXCR4 Expression

Anthony D. Cristillo,* Xiaodong Xiao,† Regina Campbell-Malone,* Dimiter S. Dimitrov,† and Barbara E. Bierer*[,1]

**Laboratory of Lymphocyte Biology, National Heart, Lung and Blood Institute, NIH, and †NCI-FCRDC, NIH, Bethesda, Maryland 20892*

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CXCR4, a G-protein-coupled chemokine receptor and HIV coreceptor, has been shown to play a central role in both chemotaxis and HIV-1-entry into T lymphocytes. Recent efforts have focused on identifying the signaling pathways that modulate CXCR4 expression in order to modulate HIV infectivity. Toward this effort, we previously demonstrated cAMP-dependent up-regulation of CXCR4 mRNA and protein in human peripheral blood T cells (PBL), resulting in increased HIV infectivity. Regulation of CXCR4 mRNA was mediated, in part, by a CRE element within the CXCR4 promoter. In order to develop a model system to examine cAMP regulation, the responses of the T lymphoblastoid cell line CEM were compared to those of human PBL. In sharp contrast to that of human PBL, HIV-1 entry into CEM cells was dramatically reduced in response to dibutyryl cAMP (DcAMP). Furthermore, while total cellular and cell surface CXCR4 protein levels were up-regulated in human PBL and in Jurkat T cells in response to DcAMP or forskolin stimulation, CXCR4 levels were unchanged by stimulation in CEM cells. Surprisingly, the CXCR4 promoter (nucleotides −1098 to +59) fused to luciferase was found to be activated similarly in CEM and Jurkat cells in response to DcAMP in a concentration-dependent manner. RT–PCR analyses confirmed that CXCR4 mRNA levels were increased by cAMP agonists. Taken together, our findings suggest that total and cell surface CXCR4 protein expression is regulated differently in human PBL than in CEM cells, a finding that correlates with the differential HIV-1 fusion in response to cAMP signaling. Moreover, our results suggest that, for CXCR4 expression and HIV viral infectivity, CEM cells may not be a faithful model of primary human lymphocytes.

Key Words: CXCR4; HIV; cAMP; CREB; chemokine; chemokine receptors; AIDS.

INTRODUCTION

CXCR4, a seven-transmembrane-spanning chemokine receptor, is coupled to and signals through a heterotrimeric GTP binding protein (Murphy, 1994). Expressed on naïve T cells, B cells, monocytes, and neutrophils (Forster *et al.*, 1998), CXCR4 binds to its ligand, stromal cell-derived factor 1-α, and induces neutrophils and lymphocyte chemotaxis (Aiuti *et al.*, 1997; Bleul *et al.*, 1996; Kim and Broxmeyer, 1998). CXCR4 has also been shown to play a critical role in the preliminary signaling events of HIV fusion and T cell entry (Alkhatib *et al.*, 1996; Choe *et al.*, 1996; Deng *et al.*, 1996; Doranz *et al.*, 1996; Dragic *et al.*, 1996; Feng *et al.*, 1996). Together with CD4, CXCR4 forms a complex with the HIV gp120 molecule and mediates infection of T-cell-tropic HIV strains (Lapham *et al.*, 1996).

The level of CXCR4 expression is correlated with HIV viral entry, and therefore definition of the regulation of CXCR4 transcription, protein translation, and surface expression is important. IL-2 has been shown to enhance CXCR4 mRNA expression (Loetscher *et al.*, 1996); several transcription factors that function as positive (NRF-1, Sp1, USF/c-Myc) and negative (YY1) regulators of CXCR4 gene expression (Moriuchi *et al.*, 1999, 1997; Wegner *et al.*, 1998) have been identified. We and others have previously reported that cAMP signaling pathways also regulate CXCR4 cell surface expression and HIV-1 infectivity (Cole *et al.*, 1999; Cristillo *et al.*, 2002). We demonstrated that cAMP-dependent CXCR4 modulation is mediated, in part,

[1] To whom correspondence and reprint requests should be addressed at National Heart, Lung and Blood Institute, Building 10, Room 6C208, 10 Center Drive, Bethesda, MD 20892. Fax: (301) 480-1792. E-mail: biererb@nih.gov.

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by a cAMP-responsive element (CRE)[2] site within the CXCR4 promoter (Cristillo *et al.*, 2002).

To develop a model of cAMP-dependent regulation of CXCR4 expression, we compared the response of human PBL to that of the T lymphoblastoid cell line CEM. We were surprised to find that HIV-1 fusion was dramatically reduced in CEM relative to human PBL in response to cAMP signaling. While both total and cell surface CXCR4 protein expression was upregulated in human PBL and Jurkat T cells after DcAMP or forskolin stimulation, CXCR4 expression was unchanged in stimulated CEM. However, DcAMP induced CXCR4 transcription in CEM cells, as in human PBL and Jurkat cells. Our results suggest that the decrease in HIV fusion following treatment with cAMP agonists is secondary to posttranscriptional inhibition of CXCR4 protein expression. Moreover, CEM cells may not be an appropriate model system for studying certain T cell signaling pathways that regulate CXCR4 protein expression and HIV viral fusion in primary human lymphocytes.

MATERIALS AND METHODS

Cells and Reagents

Human peripheral blood lymphocytes (PBL) were obtained from healthy human donors, isolated by apheresis followed by reverse flow elutriation and Ficoll–Hypaque centrifugation, and washed with 1× phosphate-buffered saline (PBS). PBL were resuspended in RPMI 1640 (MediaTech, Herndon, VA) supplemented with 10% heat-inactivated fetal calf serum (FCS) (GIBCO-BRL, Life Technologies, Gaithersburg, MD), 2 mM L-glutamine, 10 mM Hepes, pH 7.2, 100 U/ml of penicillin, 100 μg/ml of streptomycin (MediaTech), and 50 μM 2-mercaptoethanol (Bio-Rad, Hercules, CA), termed 10% RPMI, and incubated at 37°C, 5% CO_2 in air. After overnight incubation, cells were stimulated as indicated with dibutyryl cAMP (DcAMP, Sigma, St. Louis, MO), forskolin (Calbiochem, La Jolla, CA), phorbol 12-myristate 13-acetate (PMA; Calbiochem, La Jolla, CA), and/or ionomycin (Iono; Calbiochem, La Jolla, CA) as indicated. The Jurkat T cell leukemia cell line was a gift of K. Smith (Cornell University, New York, NY). The T cell lymphoblastoid leukemia cell line, CEM, was a gift of Hana Golding (Division of Viral Products, Center for Biologics Evaluation and Research, FDA Bethesda, MD).

[2] Abbreviations used: 10% cRPMI, complete RPMI media plus 10% FBS; CRE, cAMP-responsive element; CREB, cAMP-responsive element binding protein; DcAMP, dibutyryl cAMP; HIV, human immunodeficiency virus; PBL, peripheral blood T lymphocytes; EMSA, electrophoretic mobility shift assay; PMA, phorbol 12-myristate 13-acetate.

HIV Cell Fusion Assay

Fusion was assayed using a reporter gene enzyme (β-gal) as outlined (Nussbaum *et al.*, 1994). Briefly, equal numbers (10^5) of target cells (stimulated human PBL or CEM infected with a vaccinia virus-encoded bacteriophage T7 RNA polymerase) and effector cells (TF228 that constitutively express HIV gp120 and were infected with vaccinia-encoded *Escherichia coli* lacZ gene linked to the T7 promoter) were mixed in a 96-well plate and kept at 37°C. Fusion by β-gal was measured 4 h later and syncytia were counted 12 h after mixing the cells. Stimulated target cells were combined, in triplicate, with effector cells and the triplicate β-gal levels were recorded and shown graphically as a mean value with standard deviation.

Western Blotting

After stimulation of human PBL as indicated, samples were centrifuged at 466*g* for 5 min and washed once with RPMI 1640. Cells were resuspended in 1× sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.01% bromophenol, 1% 2-mercaptoethanol). Samples were vortexed and boiled for 5 min at 95°C. Proteins were separated by SDS–PAGE (Protogel, National Diagnostics, Atlanta, GA). Electrophoresed proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA), immunoblotted with a rabbit antihuman CXCR4 antibody (QED Bioscience Inc., San Diego, CA), and detected by enhanced chemiluminescence (ECL) (Amersham, Arlington Heights, IL) according to the manufacturer's instructions.

Cell Surface Staining

Human PBL, stimulated as described, were harvested by centrifugation for 5 min at 500*g*. For cell surface staining, cells were resuspended in 1× PBS and incubated with the phycoerythrin-conjugated mouse antihuman CXCR4 antibody (12G5; R & D Systems, Minneapolis, MN) or isotype control antibody for 30 min at 4°C in the dark. After 30 min, cells were washed twice with 1× PBS, resuspended in 1% paraformaldehyde (in 1× PBS), and analyzed by FACS using a Coulter cytometer.

Constructs and Transient Transfections

pGL-CXCR4 (−1098 to +59) was a generous gift from H. Moriuchi (Department of Pediatrics, Nagasaki University School of Medicine, Nagasaki, Japan) and A. Fauci (NIAID, NIH, Bethesda, MD) and was previously described (Cristillo *et al.*, 2002; Moriuchi *et al.*, 1997). Firefly luciferase reporter constructs were cotransfected with a reporter vector that contained a cDNA encoding *Renilla* luciferase (pRL-TK) under the control of the herpes simplex virus thymidine kinase promoter (Promega, Madison, WI). pRL-TK was used to control for transfection efficiency. Human PBL or CEM (10^7 cells) were transfected with 50 μg of firefly luciferase construct and 1 μg of *Renilla* luciferase construct by electroporation (320 V and 1180 μF, Cell Porator Life Technologies, Gaithersburg, MD). Cells were incubated for 24 h at 37°C, 5% CO_2 and then stimulated as described. The dual luciferase assay (Promega, Madison, WI) was performed to determine both firefly and *Renilla* luciferase activities in cell lysates. Briefly, stimulated cell suspensions were transferred to Eppendorf tubes and pelleted by centrifugation at 500*g* for 5 min. Cell pellets were washed once with 1× PBS and then lysed with 50 μl of 1× Promega passive lysis buffer. Samples were vortexed for 30 s, incubated at RT for 15 min, and pelleted again 5 min at 20,000*g*. The luminescence of 100 μl of luciferase assay reagent added to 20 μl of each lysate was recorded using a Lamat LB9507 luminometer (EG&G Berthold, Gaithersburg, MD). Finally, 100 μl of Stop & Glo reagent was added to the sample and a second luminescence reading recorded (*Renilla* luciferase).

RT–PCR

Total RNA was prepared from human PBL using Trizol (Life Technologies/GIBCO-BRL, Rockville, MD) and quantitated using OD_{260} and the RiboGreen RNA quantitation kit (Molecular Probes, Eugene, OR). mRNA levels were assayed using the Onestep RT–PCR kit (Qiagen, Valencia, CA) using the following OPC-purified primers (BioServe Biotechnologies, Laurel, MD): CXCR4-F, 5′-ATC TGG AGA ACC AGC GGT TAC C-3′; CXCR4-R, 5′-GCA GCC TGT ACT TGT CCG TCA-3′; β-actin-F, 5′-ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3′; β-actin-R, 5′-CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC-3′; where F is forward and R is reverse. RT–PCR was performed using the following conditions: 50°C for 30 min, 95°C for 15 min, 30 cycles of (i) 94°C for 1 min (ii) 55°C for 1 min, and (iii) 72°C for 1 min, and 72°C for 10 min. Samples were analyzed by gel electrophoresis and bands were revealed by staining gels with ethidium bromide. Bands were quantitated by phosphoimaging analysis using ImageQuant software and mRNA levels were normalized to β-actin mRNA levels as indicated.

RESULTS

Differential HIV-1 Fusion in Human Peripheral Blood T Cells and CEM Treated with Dibutyryl cAMP

To evaluate the effect of cAMP and other T cell signaling pathways on HIV-1 viral entry, we used a recombinant vaccinia virus-based assay to measure activation of a reporter gene (β-galactosidase) upon fusion of two distinct cell populations (Fig. 1). Target cells (stimulated human PBL or CEM), infected with a vaccinia virus-encoded bacteriophage T7 RNA polymerase, were mixed with effector cells (TF228) that constitutively express HIV gp120 and vaccinia-encoded *E. coli* lacZ gene linked to the T7 promoter. Cell fusion was then assayed by measuring β-gal activity. Stimulation of human PBL by an agent that activates protein kinase C directly (PMA) minimally enhanced cell fusion, whereas stimulation by PMA in the presence of the calcium ionophore ionomycin and by the cAMP agonist DcAMP did not modulate cell fusion (Fig. 1A). In contrast, treatment of CEM cells with PMA, PMA plus ionomycin, or DcAMP (10 mM) decreased HIV-1 viral fusion (Fig. 1B). The decrease in HIV-1 viral fusion was concentration-dependent, as 0.1 nM DcAMP was ineffective. Syncytia formation following DcAMP treatment was similarly decreased in CEM cells (data not shown) but not in human PBL (Cristillo *et al.*, 2002). Our results suggest that the signaling pathways that result in HIV-1 viral fusion differ between human PBL and CEM cells.

Differential CXCR4 Protein Expression by cAMP Agonists

We have recently observed that, in human PBL, cAMP-dependent increases in HIV infectivity correlated with cAMP-dependent transcriptional activation of CXCR4 mRNA and increased CXCR4 protein expression on the cell surface (Cristillo *et al.*, 2002). In order to assess whether the cAMP-dependent inhibition of HIV-1 viral fusion noted in CEM cells correlated with CXCR4 protein expression, we examined CXCR4 protein expression following DcAMP and forskolin treatment. Cells were treated over time with

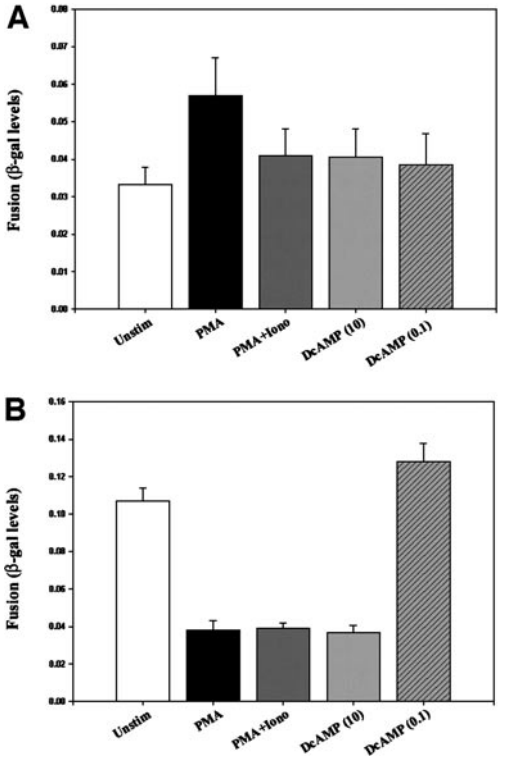


FIG. 1. Decreased HIV-1 fusion in CEM cells treated with dibutyryl cAMP. Human peripheral blood T lymphocytes (PBL) (A) and CEM cells (B) were left unstimulated or stimulated with PMA (10 ng/ml), PMA plus ionomycin (1 μM), or DcAMP (10 and 0.1 mM) for 40 h. HIV-1 viral fusion was measured as described (Materials and Methods). Stimulated target cells (human PBL or CEM) were combined with effector cells in triplicate; β-gal levels were recorded and are shown graphically as a mean value with standard deviation. The experiment was repeated three times with similar results.

drug, cell lysates were prepared, proteins separated by electrophoresis, and total CXCR4 protein was determined by Western blot analysis of whole cell lysates. In human PBL, as expected (Cole *et al.*, 1999; Cristillo *et al.*, 2002), CXCR4 protein levels increased in response to DcAMP treatment in a time-dependent manner over the 24-h period examined (Figs. 2A, left, and 2B). Conversely, CEM cells similarly stimulated with DcAMP demonstrated no measurable change in CXCR4 levels (Figs. 2A, right, and 2B). The difference in CXCR4 protein expression to DcAMP between human PBL and CEM was confirmed using forskolin, an activator of adenylate cyclase that leads to increased intracellular cAMP levels. Forskolin treatment of human PBL, but not CEM, increased CXCR4 protein expression (Fig. 2B). Our findings suggest that CXCR4 protein expression in response to cAMP agonists differs between human PBL and CEM, consistent with the difference in HIV-1 viral fusion noted.

cAMP-Dependent Regulation of CXCR4 Cell Surface Expression

We next examined whether changes in cAMP concentration modified the cell surface CXCR4 expression in CEM cells, as it had in human PBL. To this end, we stimulated human PBL, CEM, and the T cell line Jurkat with increasing concentrations of DcAMP; subsequently, we used direct immunofluorescence and flow cytometry to determine CXCR4 protein expression in intact cells. As expected, CXCR4 cell surface protein expression on human PBL stimulated for 24 h (Fig. 3A) and for 36 h (Fig. 3B) with 0.1 mM DcAMP was increased compared to that on unstimulated cells (Fig. 3A, left). CXCR4 surface expression was further increased in response to DcAMP at 1 and 10 mM concentrations (Figs. 3A and 3B, left). No change in CXCR4 cell surface expression on CEM cells was noted following DcAMP treatment (Figs. 3A and 3B, middle panels). To eliminate the possibility that the difference in cAMP regulation of CXCR4 protein expression was secondary to proliferation and cell division, the T cell line Jurkat was used. Like CEM cells, Jurkat T cells are continuously proliferating. CXCR4 protein expression was increased at both 24 and 36 h following DcAMP treatment of Jurkat cells (Figs. 3A and 3B, right), like human PBL. As the Jurkat cell line used here does not express CD4 (data not shown), we were unable to test HIV-1 fusion in response to DcAMP.

Responsiveness of CXCR4 Promoter and mRNA Levels to cAMP Signaling

To determine whether the inability of DcAMP to induce CXCR4 protein or cell surface expression in CEM cells was mediated at the level of transcription, CXCR4 promoter activity (Fig. 4) and mRNA levels (Fig. 5) were assayed. CEM and Jurkat cells were transiently transfected cells with a molecular construct containing the CXCR4 promoter sequence (-1098 to $+59$) fused to the firefly luciferase gene. Following overnight rest, cells were stimulated and luciferase activity was determined at 12 h. Luciferase activity was normalized to control for the level of transfection. There was a dose-dependent increase in CXCR4 promoter activity induced by DcAMP in both Jurkat (Fig. 4A) and CEM (Fig. 4B) cells relative to unstimulated samples. This increase was consistent with that observed in human PBL

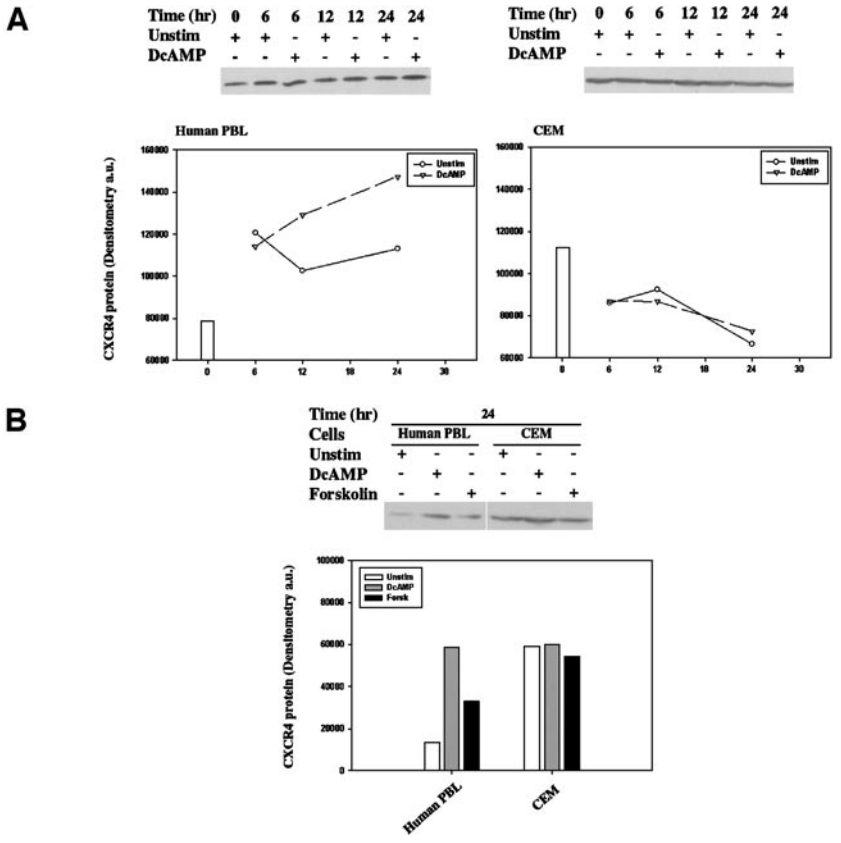
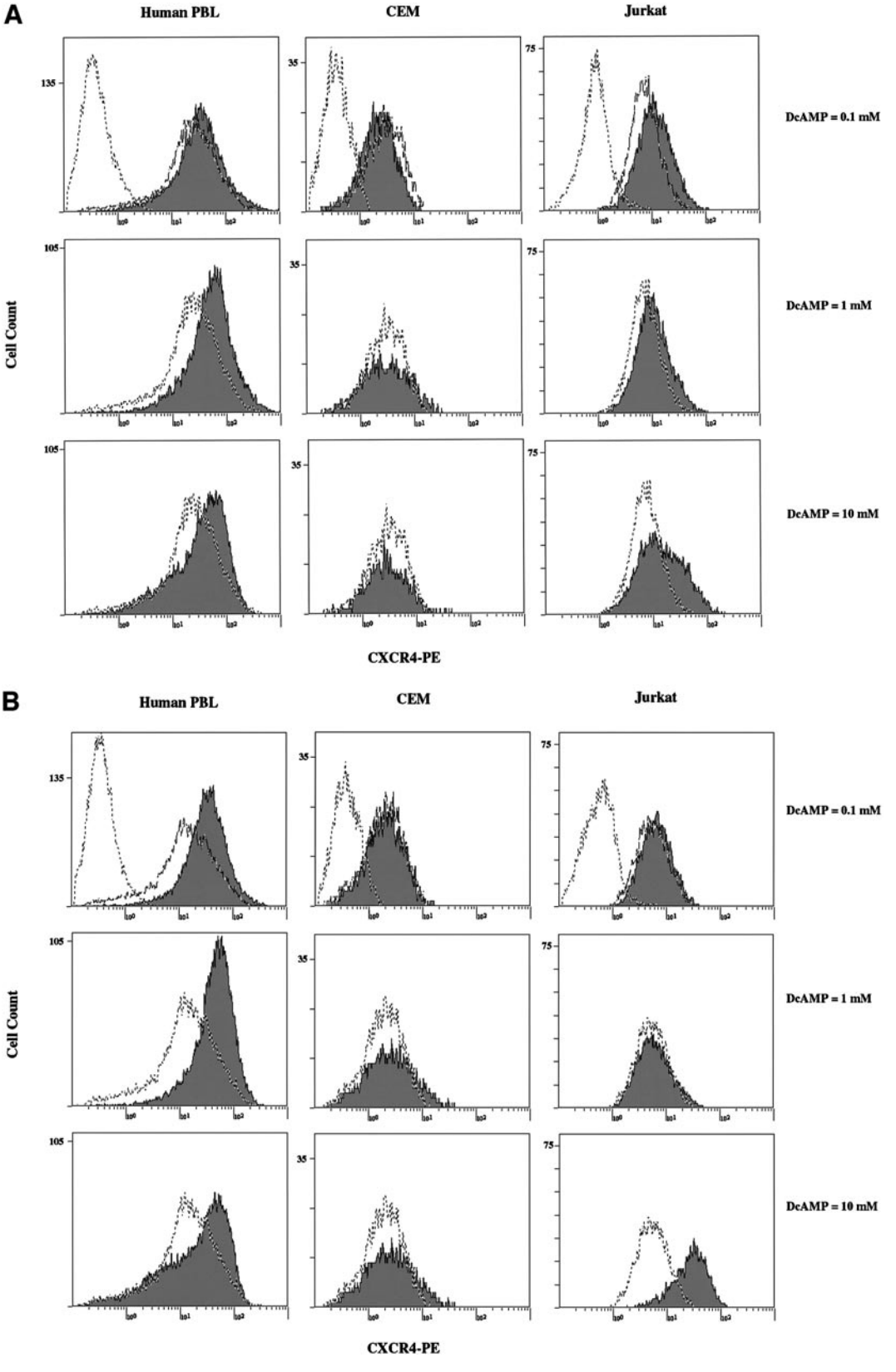


FIG. 2. Unchanged CXCR4 protein expression in DcAMP or forskolin-stimulated CEM cells. CXCR4 protein levels in human PBL and CEM were assessed by an immunoblotting assay. Cells were treated for 0, 6, 12, and 24 h as described with ethanol diluent, DcAMP (A, B), or forskolin (B). Cells were lysed (see Materials and Methods) and proteins were separated by SDS–PAGE, transferred to a PVDF membrane, and immunoblotted with a rabbit antihuman CXCR4 antibody. The CXCR4 band was detected by ECL. Bands were quantitated and values expressed in arbitrary units (a.u.) using ImageQuant software and are graphically represented.

(Cristillo *et al.*, 2002). In addition, CXCR4 mRNA levels were upregulated in both Jurkat and CEM cells in response to DcAMP and forskolin (Fig. 5), confirming that cAMP agonists are able to activate the CXCR4 promoter and induce transcriptional activation of CXCR4 mRNA. Taken together, our data suggest that, despite cAMP-dependent induction of CXCR4 mRNA, CXCR4 protein levels are not increased intracellularly nor on the cell surface on CEM cells, unlike in human peripheral blood lymphocytes.

DISCUSSION

Chemokine receptors constitute a large family of G-protein-coupled receptors that modulate various biological processes, such as leukocyte trafficking, hematopoesis, and angiogenesis, by binding to chemotactic cytokines (Locati and Murphy, 1999; Melchers *et al.*, 1999; Zlotnik and Yoshie, 2000). Five chemokine receptor classes, CC, CXC, CC/CXC, C, and CX3C, have been described and, within the CXC class, CXCR4 has been identified as the principal coreceptor for entry of T-tropic (X4) HIV-1 virus into T lymphocytes (Feng *et al.*, 1996; Schuitemaker *et al.*, 1992; Simmons *et al.*, 1996). The natural ligand for CXCR4, stromal cell-derived factor-1 alpha (SDF-1α, CXCL12), was reported to be highly expressed in fetal liver and bone marrow stromal cells (Bleul *et al.*, 1996; Oberlin *et al.*, 1996; Zlotnik and Yoshie, 2000). In contrast to other chemokines that can bind to and signal through a number of individual chemokine receptors, SDF-1α binds to and signals through CXCR4 alone.


A
Human PBL
CEM
Jurkat
DcAMP = 0.1 mM
DcAMP = 1 mM
DcAMP = 10 mM
Cell Count
CXCR4-PE
B
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CEM
Jurkat
DcAMP = 0.1 mM
DcAMP = 1 mM
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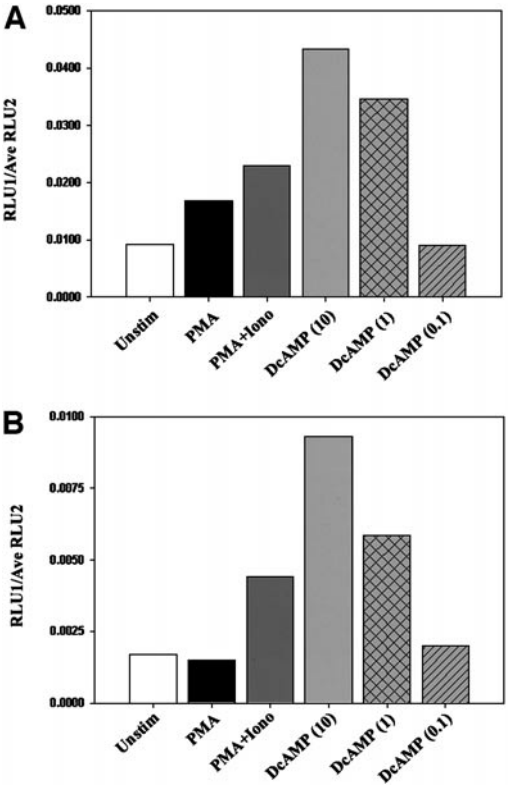

FIG. 4. Increased CXCR4 promoter activity by DcAMP in transiently transfected Jurkat and CEM cells. CXCR4 promoter–luciferase constructs were cotransfected with pRL-TK into human PBL (10^7 cells) as described under Materials and Methods. After a 24-h incubation, Jurkat (A) or CEM (B) cells (10^6 cells/sample) were incubated in ethanol diluent control, PMA (10 ng/ml) (black bars), PMA plus ionomycin (Iono, 1 μM) (dark gray bars), DcAMP (10 mM, solid light gray bars), DcAMP (1 mM, hatched light gray bars), and DcAMP (0.1 mM, striped light gray bars) as indicated. Twelve hours later, cells were harvested and luciferase activity was determined. Firefly luciferase (RLU1) was normalized to the average value of *Renilla* luciferase values obtained (average RLU2 values) to control for transfection efficiency. Representative experiments are shown that were reproducible in at least three experiments.

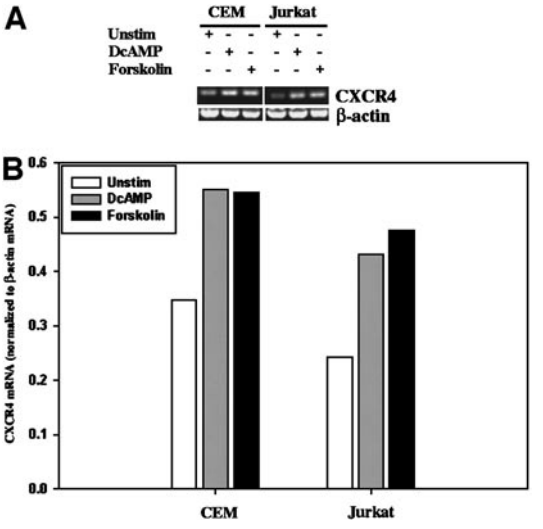

FIG. 5. DcAMP- and forskolin-induced CXCR4 mRNA expression in Jurkat and CEM cells. Jurkat and CEM cells were either left unstimulated or treated for 3 h with DcAMP or forskolin as indicated. Total RNA was prepared and quantitated as described (Materials and Methods). RT-PCR was performed as outlined (Materials and Methods) using CXCR4- and β-actin-specific primers. Samples were analyzed by gel electrophoresis and bands were revealed by staining with ethidium bromide (A). Bands were quantitated by phosphoimaging analysis using ImageQuant software and mRNA levels were normalized to β-actin mRNA levels and graphically represented (B). One representative experiment of at least three reproducible experiments is shown.

An understanding of the regulatory mechanisms governing CXCR4 expression may be useful for designing therapeutic strategies to control HIV entry and viral replication in T lymphocytes. To this end, SDF-1α and phorbol esters have been shown to down-modulate cell surface CXCR4 protein. Receptor internalization by phorbol esters was attributed to protein kinase C activation and phosphorylation of the carboxy-terminal serine amino acids of CXCR4 (Amara *et al.*, 1997; Signoret *et al.*, 1997). Studies of the transcriptional regulation of CXCR4 revealed both positive and negative transcriptional regulators of CXCR4 gene expression (Cristillo *et al.*, 2002; Moriuchi *et al.*, 1999, 1997; Wegner *et al.*, 1998). We (Cristillo *et al.*, 2002) and others (Cole *et al.*, 1999) recently reported a cAMP-dependent increase in CXCR4 cell surface expression and HIV infectivity in human peripheral blood T cells. We found that

FIG. 3. Increased CXCR4 cell surface expression in human PBL and Jurkat cells and unchanged expression in CEM cells by DcAMP. Human PBL, CEM, and Jurkat cells were treated in the presence and the absence of DcAMP (0.1, 1, and 10 mM) as indicated for 24 h (A) or 36 h (B). Cells were labeled with phycoerythrin-conjugated antihuman CXCR4 mAb as described (Materials and Methods) to quantitate cell surface CXCR4 expression. Cell surface CXCR4 expression following DcAMP treatment of human PBL (left), CEM cells (middle), and Jurkat cells (right) is shown. For simplicity, the isotype control is shown (dotted line/unshaded) in the top panel only of each cell line. The top, middle, and bottom panels show representative histograms of CXCR4-specific fluorescence comparing unstimulated (solid lines/unshaded) and 0.1, 1.0, and 10 mM DcAMP (solid lines/shaded)-treated samples as indicated.

cAMP-dependent CXCR4 up-regulation is mediated by binding of the cAMP-responsive element binding protein-1 (CREB-1) to a CRE site within the CXCR4 promoter. Here, we extend our current understanding by demonstrating that HIV-1 fusion and CXCR4 protein expression are regulated differently in CEM cells than in human PBL. We describe a cAMP-dependent increase in CXCR4 promoter activity and mRNA levels in CEM cells that did not translate into an increase in either total or cell surface protein expression, unlike in human PBL.

Total CXCR4 protein levels did not appear to increase in CEM cells in response to DcAMP or forskolin stimulation (Fig. 2). Taken together, these findings suggest that cAMP signaling increased CXCR4 mRNA levels but the mRNA was not translated into CXCR4 protein. It is possible, however, that a CXCR4 protein product is generated that has a shorter half-life following a rise in intracellular cAMP levels in CEM cells compared to human PBL. Thus, cAMP signaling pathways in CEM cells might induce CXCR4 promoter activity (Fig. 4), mRNA transcription (Fig. 5), and translation, but rapid protein turnover would result in no net change in protein expression (Fig. 2). Either possibility suggests a difference between CEM cells and human PBL in cAMP-dependent protein synthesis (or degradation) pathways.

We considered the possibility that CEM cells expressed a variant of the 1.7-kb CXCR4 mRNA transcript termed CXCR4-Lo (Gupta and Pillarisetti, 1999) and that this variant was regulated differently than CXCR4. CXCR4-Lo is the protein product of a larger 4.0-kb transcript predominantly expressed in PBL and spleen (Gupta and Pillarisetti, 1999). We did not detect expression of CXCR4-Lo in either CEM or human PBL using appropriate primers that distinguish CXCR4 from CXCR4-Lo by RT–PCR (data not shown). Thus, cell type differences in CXCR4 isoform expression do not explain the differences between CEM cells on the one hand and PBL and Jurkat cells on the other.

In CEM cells, cAMP-dependent stimulation did not alter CXCR4 cell surface protein expression at 24 h (Fig. 3A) or 36 h (Fig. 3B) following cell treatment (Fig. 3 and data not shown); in contrast, in human PBL and Jurkat T cells, cAMP agonists increased CXCR4 cell surface expression (Fig. 3). While this differential cell surface expression correlated with the differences in cell type in HIV fusion (Fig. 1), other receptors, in addition to CXCR4, may also affect HIV viral fusion. To this end, we verified that CD4 cell surface expression was not modulated by DcAMP stimulation on either human PBL or CEM cells (data not shown). Adhesion receptors (e.g., LFA-1, CD54) (Golding *et al.*, 1992; Gruber *et al.*, 1991; Hioe *et al.*, 2001), costimulatory receptors (e.g., CD26) (Callebaut *et al.*, 1998), and soluble mediators (e.g., IL-16, SDF-1α) (Marechal *et al.*, 1999; Zhou *et al.*, 1999), among others, have all been correlated with HIV fusion and syncytia formation in other systems and may contribute to the differences observed here.

CEM cells represent a $CD4^+$, $CXCR4^+$ T lymphoblastoid cell line that has been extensively used as a model system for studying HIV fusion and viral entry (Callebaut *et al.*, 1998; Dimitrov *et al.*, 1991; Fujita *et al.*, 1992; Hesselgesser *et al.*, 1998; Jiang *et al.*, 1997; Schols *et al.*, 1990). Our study demonstrates that cAMP signaling pathways can differentially affect both CXCR4 protein expression and HIV-1 fusion in CEM and human primary lymphocytes. While the mechanism(s) mediating the inability of CEM to increase CXCR4 protein levels coordinately with the promoter activity is at present obscure, we are now cautious in our use of CEM cells to predict and model the response of primary human lymphocytes in the study of HIV viral fusion and receptor expression. Whether this concern extends beyond the cAMP signaling pathway will be appreciated when appropriate correlative studies to primary human T cells are performed.

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REFERENCES

Aiuti, A., Webb, I. J., Bleul, C., Springer, T., and Gutierrez-Ramos, J. C. (1997). The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J. Exp. Med.* **185,** 111–201.

Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M., and Berger, E. A. (1996). CC CKR5: A RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272,** 1955–1958.

Amara, A., Gall, S. L., Schwartz, O., Salamero, J., Montes, M., Loetscher, P., Baggiolini, M., Virelizier, J. L., and Arenzana-Seisdedos, F. (1997). HIV coreceptor downregulation as antiviral principle: SDF-1alpha-dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J. Exp. Med.* **186,** 139–146.

Bleul, C. C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroski, J., and Springer, T. A. (1996a). The lymphocyte chemoattractant SDF-1

is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* **382,** 829–833.

Bleul, C. C., Fuhlbrigge, R. C., Casasnovas, J. M., Aiuti, A., and Springer, T. A. (1996b). A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1) [see comments]. *J. Exp. Med.* **184,** 1101–1109.

Callebaut, C., Jacotot, E., Blanco, J., Krust, B., and Hovanessian, A. G. (1998). Increased rate of HIV-1 entry and its cytopathic effect in CD4+/CXCR4+ T cells expressing relatively high levels of CD26. *Exp. Cell Res.* **241,** 352–362.

Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C., and Sodroski, J. (1996). The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85,** 1135–1148.

Cole, S. W., Jamieson, B. D., and Zack, J. A. (1999). cAMP up-regulates cell surface expression of lymphocyte CXCR4: Implications for chemotaxis and HIV-1 infection. *J. Immunol.* **162,** 1392–1400.

Cristillo, A. D., Highbarger, H. C., Dewar, R. L., Dimitrov, D. S., Golding, H., and Bierer, B. E. (2002). Up-regulation of HIV coreceptor CXCR4 expression in human T lymphocytes is mediated in part by a cAMP-responsive element. *FASEB J.* **16,** 354–364.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Landau, N. R. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381,** 661–666.

Dimitrov, D. S., Golding, H., and Blumenthal, R. (1991). Initial stages of HIV-1 envelope glycoprotein-mediated cell fusion monitored by a new assay based on redistribution of fluorescent dyes. *AIDS Res. Hum. Retroviruses* **7,** 799–805.

Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G., and Doms, R. W. (1996). A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85,** 1149–1158.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, J. P., and Paxton, W. A. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5 [see comments]. *Nature* **381,** 667–673.

Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272,** 872–877.

Forster, R., Kremmer, E., Schubel, A., Breitfeld, D., Kleinschmidt, A., Nerl, C., Bernhardt, G., and Lipp, M. (1998). Intracellular and surface expression of the HIV-1 coreceptor CXCR4/fusin on various leukocyte subsets: Rapid internalization and recycling upon activation. *J. Immunol.* **160,** 1522–1531.

Fujita, K., Silver, J., and Peden, K. (1992). Changes in both gp120 and gp41 can account for increased growth potential and expanded host range of human immunodeficiency virus type 1. *J. Virol.* **66,** 4445–4451.

Golding, H., Dimitrov, D. S., and Blumenthal, R. (1992). LFA-1 adhesion molecules are not involved in the early stages of HIV-1 env-mediated cell membrane fusion. *AIDS Res. Hum. Retroviruses* **8,** 1593–1598.

Gruber, R., Endl, J., Froschl, M., Rieber, E. P., Ziegler-Heitbrock, H. W., and Riethmuller, G. (1991). Quantitative analysis of CD6, CD4 and CD8 cell surface molecules compared to the absolute numbers of CD6+, CD4+ and CD8+ T-cells in peripheral blood in patients with HIV-infection. *J. Clin. Lab. Immunol.* **35,** 157–163.

Gupta, S. K., and Pillarisetti, K. (1999). Cutting edge: CXCR4-Lo: Molecular cloning and functional expression of a novel human CXCR4 splice variant. *J. Immunol.* **163,** 2368–2372.

Hesselgesser, J., Liang, M., Hoxie, J., Greenberg, M., Brass, L. F., Orsini, M. J., Taub, D., and Horuk, R. (1998). Identification and characterization of the CXCR4 chemokine receptor in human T cell lines: Ligand binding, biological activity, and HIV-1 infectivity. *J. Immunol.* **160,** 877–883.

Hioe, C. E., Chien, P. C., Jr., Lu, C., Springer, T. A., Wang, X. H., Bandres, J., and Tuen, M. (2001). LFA-1 expression on target cells promotes human immunodeficiency virus type 1 infection and transmission. *J. Virol.* **75,** 1077–1082.

Jiang, J. D., Wilk, S., Li, J., Zhang, H., and Bekesi, J. G. (1997). Inhibition of human immunodeficiency virus type 1 infection in a T-cell line (CEM) by new dipeptidyl-peptidase IV (CD26) inhibitors. *Res. Virol.* **148,** 255–266.

Kim, C. H., and Broxmeyer, H. E. (1998). In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: Stromal cell-derived factor-1, steel factor, and the bone marrow environment. *Blood* **91,** 100–110.

Lapham, C. K., Ouyang, J., Chandrasekhar, B., Nguyen, N. Y., Dimitrov, D. S., and Golding, H. (1996). Evidence for cell-surface association between fusin and the CD4-gp120 complex in human cell lines. *Science* **274,** 602–605.

Locati, M., and Murphy, P. M. (1999). Chemokines and chemokine receptors: biology and clinical relevance in inflammation and AIDS. *Annu. Rev. Med.* **50,** 425–440.

Loetscher, P., Seitz, M., Baggiolini, M., and Moser, B. (1996). Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. *J. Exp. Med.* **184,** 569–577.

Marechal, V., Arenzana-Seisdedos, F., Heard, J. M., and Schwartz, O. (1999). Opposite effects of SDF-1 on human immunodeficiency virus type 1 replication. *J. Virol.* **73,** 3608–3615.

Melchers, F., Rolink, A. G., and Schaniel, C. (1999). The role of chemokines in regulating cell migration during humoral immune responses. *Cell* **99,** 351–354.

Moriuchi, M., Moriuchi, H., Margolis, D. M., and Fauci, A. S. (1999). USF/c-Myc enhances, while Yin-Yang 1 suppresses, the promoter activity of CXCR4, a coreceptor for HIV-1 entry. *J. Immunol.* **162,** 5986–5992.

Moriuchi, M., Moriuchi, H., Turner, W., and Fauci, A. S. (1997). Cloning and analysis of the promoter region of CXCR4, a coreceptor for HIV-1 entry. *J. Immunol.* **159,** 4322–4329.

Murphy, P. M. (1994). The molecular biology of leukocyte chemoattractant receptors. *Annu. Rev. Immunol.* **12,** 593–633.

Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J. L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J. M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggiolini, M., and Moser, B. (1996). The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* **382,** 833–835.

Schols, D., Pauwels, R., Desmyter, J., and De Clercq, E. (1990). Flow cytometric method to monitor the destruction of CD4+ cells following their fusion with HIV-infected cells. *Cytometry* **11,** 736–743.

Schuitemaker, H., Koot, M., Kootstra, N. A., Dercksen, M. W., de Goede,

R. E., van Steenwijk, R. P., Lange, J. M., Schattenkerk, J. K., Miedema, F., and Tersmette, M. (1992). Biological phenotype of human immunodeficiency virus type 1 clones at different stages of infection: Progression of disease is associated with a shift from monocytotropic to T-cell-tropic virus population. *J. Virol.* **66,** 1354–1360.

Signoret, N., Oldridge, J., Pelchen-Matthews, A., Klasse, P. J., Tran, T., Brass, L. F., Rosenkilde, M. M., Schwartz, T. W., Holmes, W., Dallas, W., Luther, M. A., Wells, T. N., Hoxie, J. A., and Marsh, M. (1997). Phorbol esters and SDF-1 induce rapid endocytosis and down modulation of the chemokine receptor CXCR4. *J. Cell. Biol.* **139,** 651–654.

Simmons, G., Wilkinson, D., Reeves, J. D., Dittmar, M. T., Beddows, S., Weber, J., Carnegie, G., Desselberger, U., Gray, P. W., Weiss, R. A., and Clapham, P. R. (1996). Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. *J. Virol.* **70,** 8355–8360.

Wegner, S. A., Ehrenberg, P. K., Chang, G., Dayhoff, D. E., Sleeker, A. L., and Michael, N. L. (1998). Genomic organization and functional characterization of the chemokine receptor CXCR4, a major entry coreceptor for human immunodeficiency virus type 1. *J. Biol. Chem.* **273,** 4754–4760.

Zhou, P., Devadas, K., Tewari, D., Jegorow, A., and Notkins, A. L. (1999). Processing, secretion, and anti-HIV-1 activity of IL-16 with or without a signal peptide in CD4+ T cells. *J. Immunol.* **163,** 906–912.

Zlotnik, A., and Yoshie, O. (2000). Chemokines: A new classification system and their role in immunity. *Immunity* **12,** 121–127.